PCT/CA 2004/001918

17 February 2005 17.02.05

PA 1278481

# THE UNITED STATES OF AMERICA

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**February 01, 2005**

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**APPLICATION NUMBER:** ***60/516,273***
**FILING DATE:** ***November 03, 2003***

**By Authority of the**
**COMMISSIONER OF PATENTS AND TRADEMARKS**

**E. BORNETT**
**Certifying Officer**

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PTO/SB/16 (02-01)
Approved for use through10/31/2002. OMB 0651-0032
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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

| INVENTOR(S) | | |
|---|---|---|
| Given Name (first and middle [if any]) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| Sanjay K. | SHARMA | Edmonton, Canada |
| Thomas | WOO | Edmonton, Canada |

☒ Additional inventors are being named on the 1 separately numbered sheets attached hereto

19587 U.S.PTO 60/516273 110303

**TITLE OF THE INVENTION (280 characters max)**

RAPAMYCIN PEPTIDES CONJUGATES: SYNTHESIS AND USES THEREOF

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

☒ Customer Number 020988 → *Place Customer Number Bar Code Label here*

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| | | | | | |
|---|---|---|---|---|---|
| ☐ Firm or Individual Name | OGILVY RENAULT | | | | |
| Address | 1981 McGill College Avenue | | | | |
| Address | Suite 1600 | | | | |
| City | Montreal | State | Quebec | ZIP | H3A 2Y3 |
| Country | Canada | Telephone | 613-780-8661 | Fax | 613-230-6706 |

**ENCLOSED APPLICATION PARTS (check all that apply)**

☒ Specification *Number of Pages* 42
☐ Drawing(s) *Number of Sheets*
☒ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☒ Other (specify) Fee Transmittal PTO/SB17

**METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT**

☐ Applicant claims small entity status. See 37 CFR 1.27.
☒ A check or money order is enclosed to cover the filing fees
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 19-5113
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

*Respectfully submitted,*

SIGNATURE _____ Date 31/10/03

TYPED or PRINTED NAME Wayne H. YAN

REGISTRATION NO. (if appropriate) 44,485

Docket Number: 15814-11USPR

TELEPHONE 613-780-8682

## USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

# *PROVISIONAL APPLICATION COVER SHEET*
*Additional Page*

PTO/SB/16 (02-01)
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| Docket Number | 15814-11USPR | Type a plus sign (+) inside this box → | + |
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**INVENTOR(S)/APPLICANT(S)**

| Given Name (first and middle [if any]) | Family or Surname | Residence (City and either State or Foreign Country) |
|---|---|---|
| Selvaraj | NAICKER | Edmonton, Canada |

Number __2__ of __2__

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## APPLICATION INFORMATION

| | |
|---|---|
| Application number:: | |
| Filing Date:: | |
| Application Type:: | Provisional |
| Title:: | **RAPAMYCIN PEPTIDES CONJUGATES: SYNTHESIS AND USES THEREOF** |
| Attorney Docket Number:: | 15814-11USPR |
| Request for Early Publication?:: | No |
| Request for Non-Publication?:: | No |
| Small Entity?:: | No |

## INVENTOR INFORMATION

| | |
|---|---|
| Inventor Authority Type:: | Inventor |
| Primary Citizenship Country:: | India |
| Status:: | Full Capacity |
| Given name:: | Sanjay |
| Middle name:: | K. |
| Family name:: | SHARMA |
| Name Suffix:: | |
| City of Residence:: | Edmonton |
| State or Province of Residence:: | Alberta |
| Country of Residence:: | Canada |
| Street:: | 204C, 3624-119 Street |
| City:: | Edmonton |
| State or Province:: | Alberta |
| Country:: | Canada |
| Postal or Zip Code:: | T6J 2X6 |

| | |
|---|---|
| Inventor Authority Type:: | Inventor |
| Primary Citizenship Country:: | Canada |
| Status:: | Full Capacity |
| Given name:: | Thomas |
| Middle name:: | |
| Family name:: | WOO |
| Name Suffix:: | |
| City of Residence:: | Edmonton |
| State or Province of Residence:: | Alberta |
| Country of Residence:: | Canada |

| | |
|---|---|
| Street:: | 130 Leigh Cr. |
| City:: | Edmonton |
| State or Province:: | Alberta |
| Country:: | Canada |
| Postal or Zip Code:: | T6R 2S8 |
| Inventor Authority Type:: | Inventor |
| Primary Citizenship Country:: | Canada |
| Status:: | Full Capacity |
| Given name:: | Selvaraj |
| Middle name:: | |
| Family name:: | NAICKER |
| Name Suffix:: | |
| City of Residence:: | Edmonton |
| State or Province of Residence:: | Alberta |
| Country of Residence:: | Canada |
| Street:: | 3304-117 Street |
| City:: | Edmonton |
| State or Province:: | Alberta |
| Country:: | Canada |
| Postal or Zip Code:: | T6J 3J4 |

**CORRESPONDENCE INFORMATION**

| | |
|---|---|
| Correspondence Customer Number:: | 020988 |
| Phone number:: | (514) 845-7126 |
| Fax:: | (514) 288-8389 |
| E-Mail Address:: | swabey@ogilvyrenault.com |

**REPRESENTATIVE INFORMATION**

Representative Customer Number:: 020988



Initial 10/30/03

**ASSIGNEE INFORMATION**

| | |
|---|---|
| Assignee name:: | Altachem Pharma Ltd. |
| Street:: | 17215-108 Avenue N.W. |
| City:: | Edmonton |
| State or Province:: | Alberta |
| Country:: | Canada |
| Postal or Zip Code:: | T5S 1G2 |



Initial 10/30/03

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# FEE TRANSMITTAL for FY 2004

*Effective 10/01/2003. Patent fees are subject to annual revision.*

☐ Applicant claims small entity status. See 37 CFR 1.27

**TOTAL AMOUNT OF PAYMENT** ($) 160

| *Complete if Known* | |
|---|---|
| Application Number | |
| Filing Date | |
| First Named Inventor | SHARMA, Sanjay K. |
| Examiner Name | |
| Art Unit | |
| Attorney Docket No. | 15814-11USPR KD/LB/sw |

## METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☑ Money Order ☐ Other ☐ None

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Deposit Account Number: 19-5113

Deposit Account Name: Ogilvy Renault

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## FEE CALCULATION

### 1. BASIC FILING FEE

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1001 | 770 | 2001 | 385 | Utility filing fee | |
| 1002 | 340 | 2002 | 170 | Design filing fee | |
| 1003 | 530 | 2003 | 265 | Plant filing fee | |
| 1004 | 770 | 2004 | 385 | Reissue filing fee | |
| 1005 | 160 | 2005 | 80 | Provisional filing fee | 160 |

SUBTOTAL (1) ($) 160

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

| | Extra Claims | | Fee from below | | Fee Paid |
|---|---|---|---|---|---|
| Total Claims | | -20** = | | X | = |
| Independent Claims | | - 3** = | | X | = |
| Multiple Dependent | | | | | = |

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description |
|---|---|---|---|---|
| 1202 | 18 | 2202 | 9 | Claims in excess of 20 |
| 1201 | 88 | 2201 | 43 | Independent claims in excess of 3 |
| 1203 | 290 | 2203 | 145 | Multiple dependent claim, if not paid |
| 1204 | 86 | 2204 | 43 | ** Reissue independent claims over original patent |
| 1205 | 18 | 2205 | 9 | ** Reissue claims in excess of 20 and over original patent |

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1051 | 130 | 2051 | 65 | Surcharge - late filing fee or oath | |
| 1052 | 50 | 2052 | 25 | Surcharge - late provisional filing fee or cover sheet | |
| 1053 | 130 | 1053 | 130 | Non-English specification | |
| 1812 | 2,520 | 1812 | 2,520 | For filing a request for *ex parte* reexamination | |
| 1804 | 920* | 1804 | 920* | Requesting publication of SIR prior to Examiner action | |
| 1805 | 1,840* | 1805 | 1,840* | Requesting publication of SIR after Examiner action | |
| 1251 | 110 | 2251 | 55 | Extension for reply within first month | |
| 1252 | 420 | 2252 | 210 | Extension for reply within second month | |
| 1253 | 950 | 2253 | 475 | Extension for reply within third month | |
| 1254 | 1,480 | 2254 | 740 | Extension for reply within fourth month | |
| 1255 | 2,010 | 2255 | 1,005 | Extension for reply within fifth month | |
| 1401 | 330 | 2401 | 165 | Notice of Appeal | |
| 1402 | 330 | 2402 | 165 | Filing a brief in support of an appeal | |
| 1403 | 290 | 2403 | 145 | Request for oral hearing | |
| 1451 | 1,510 | 1451 | 1,510 | Petition to institute a public use proceeding | |
| 1452 | 110 | 2452 | 55 | Petition to revive - unavoidable | |
| 1453 | 1,330 | 2453 | 665 | Petition to revive - unintentional | |
| 1501 | 1,330 | 2501 | 665 | Utility issue fee (or reissue) | |
| 1502 | 480 | 2502 | 240 | Design issue fee | |
| 1503 | 640 | 2503 | 320 | Plant issue fee | |
| 1460 | 130 | 1460 | 130 | Petitions to the Commissioner | |
| 1807 | 50 | 1807 | 50 | Processing fee under 37 CFR 1.17(q) | |
| 1806 | 180 | 1806 | 180 | Submission of Information Disclosure Stmt | |
| 8021 | 40 | 8021 | 40 | Recording each patent assignment per property (times number of properties) | |
| 1809 | 770 | 2809 | 385 | Filing a submission after final rejection (37 CFR 1.129(a)) | |
| 1810 | 770 | 2810 | 385 | For each additional invention to be examined (37 CFR 1.129(b)) | |
| 1801 | 770 | 2801 | 385 | Request for Continued Examination (RCE) | |
| 1802 | 900 | 1802 | 900 | Request for expedited examination of a design application | |

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 0

## SUBMITTED BY

| | | | | | |
|---|---|---|---|---|---|
| Name (Print/Type) | Wayne H. Yan | Registration No. (Attorney/Agent) | 44,485 | (Complete *(if applicable)*) Telephone | 613-780-8673 |
| Signature | | | | Date | October 31, 2003 |

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# RAPAMYCIN PEPTIDES CONJUGATES: SYNTHESIS AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This is the first application filed for the present invention.

## TECHNICAL FIELD

**[0002]** This application relates to cell cycle inhibitors. More particularly the invention relates to the synthesis rapamycin peptides conjugates and their use in treating disorders related to cell division.

## BACKGROUND OF THE INVENTION

**[0003]** Cancer drug discovery is one of the most rapidly changing areas of pharmaceutical research. Most anticancer agents that are approved for clinical use are molecules which damage deoxyribonucleic acid (DNA), block DNA synthesis indirectly through inhibition of nucleic acid precursor biosynthesis or disrupt hormonal stimulation of cell growth (Sielecki, T.M. et al. *J. Med. Chem.* 2000, 43(1), 1-18). There has been a recent shift of emphasis towards novel mechanistic targets that has emerged as a direct consequence of the intense study of the underlying genetic changes associated with the cancerous state. The high frequency of mutations in cancer cells which results in altered cell cycle regulation, in conjunction with aberrant expression of cyclin dependent kinases (CDKs) and growth signal transduction, conferring a proliferative advantage, indicates that many of these aberrant mechanisms may be strategic targets for cancer therapy. An increasing body of evidence has shown a link between tumor development and CDK related malfunctions. Over expression of the cyclic

regulatory protein and subsequent kinase hyperactivity have been linked to several types of cancers. The process of cell division has been amply studied but the molecular mechanisms that regulate the cell cycle have only been elucidate in the last two decades. The phases of the cell cycle are: The rest phase, $G_o$, active protein synthesis in preparation of cell division occurs in the $G_1$ phase. During the $G_1$ phase the volume of the cell increases. After the $G_1$ phase the cells enter the S phase in which the DNA is replicated. The S phase is followed by another gap phase, $G_2$, during which DNA replication is completed. The last phase is the mitosis or M phase in which the cells divide (Muhtasib, H.G. et al. *Curr. Cancer Drug Targets* 2002, 2, 309-336).

**[0004]** Rapamycin (Sirolimus, Rapamune, 1,18-dihydroxy-12-[2(4-hydroxy-3-methoxy-cyclohexyl)-1-methyl-ethyl]-19,30-4-aza-tricyclo[30.3.1% 4,9 &] hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone) with a molecular formula of $C_{51}H_{79}NO_{13}$ and molecular mass of 913.6 Da was isolated in 1975 from the bacteria strain *Streptomyces hygroscopicus* found in a soil sample on Ester Island (Sehgal, S.N. et al. *J. Antibiot.* 1975, 28, 721 and Sehgal, S.N. et al. *J. Antibiot.* 1975, 28, 727). Rapamycin has potent antimicrobial, immunosuppressant and antitumor properties. It inhibits the translation of key mRNAs of proteins required for the cell cycle progression from $G_1$ to S phase by binding intracellularly to the immunophilin FK506 binding protein FKBP12 and the resultant complex inhibits the protein kinase activity of a protein kinase termed mammalian target of rapamycin (mTOR). The inhibition of mTOR, in turn blocks signals to two separate downstream pathways which control the translation of specific mRNA (40S ribosomal protein S6 kinase $P70^{S6K}$) required for cell

cycle traverse from $G_1$ to S phase (Wiederrecht, G.J. et al. *Prog. Cell.Cycle. Res.* 1995, 1, 53-71).

**[0005]** The poor aqueous solubility and chemical stability of rapamycin precluded its clinical development as an anticancer agent. Recently a series of rapamycin analogs with improved aqueous solubility and stability have been synthesized and evaluated. CCI-779 (Wyeth Ayerst, PA, USA), a soluble ester analog of rapamycin is selected for development as an anti cancer agent based on its prominent antitumor profile and favourable pharmaceutical and toxicological characterstics in preclinical studies (Huang, S. et al. *Curr. Opin. Investig. Drugs* 2002, 3, 295-304). CCI-779 has demonstrated significant inhibitory effects both in vivo and in vitro (various cell lines lines with $IC_{50}$ values of $< 10^{-8}$ M). Its cytostatic properties results from the inhibition of translation of several key proteins that regulate the $G_1$ phase of the cell cycle. Similar to rapamycin, CCI-779 is hypothesized to form a complex with the intracellular cytoplasmic protein FK506 binding protein -12 (FKBP) that binds to mTOR resulting in the inhibition of key signaling pathways involved in the $G_1$ phase of the cell cycle and thereby checks the progression from $G_1$ to S phase. Studies have shown that CCI-779 is able to penetrate the blood brain barrier as it has aqueous solubility and is highly lipophilic. Phase I and II studies have shown that CCI-779 is associated predominantly with skin toxicities (rash, folliculitis, prurtis, ulceration and nail changes), stomatic and asthenia (Elit, L. *Curr Opin. Investig. Drugs* 2002, 3, 1249-1253 and Punt, C.J.A. et al. *Annals of Oncology* 2003, 14, 931-937).

**[0006]** The CDK complex activity is regulated by mechanisms such as stimulatory or inhibitory phosphorylations as well

as the synthesis and degradation of the kinase and cyclin subunits themselves. Recently a link has been established between the regulation of the activity of the cyclin dependent kinases and cancer by the discovery of a group of CDK inhibitors including $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and $p16^{Ink4/MTS1}$. The inhibitory activity of $p27^{Kip1}$ is induced by the negative growth factor TGF-β and by contact inhibition (Nurse et al. *Nature* 1994, 372(8), 570-573). The interleukin-2 (IL-2) allows CDK activation by causing the elimination of the CDK inhibitor protein $p27^{Kip1}$, and that this is prevented by rapamycin. By contrast, the CDK inhibitor p21 is induced by IL-2 and this induction is blocked by rapamycin. The activity of $p21^{Waf1/Cip1}$ is regulated transcriptionally by DNA damage through the induction of p53, senesence and quiesence. The tumor suppressor protein $p21^{Waf1}$ plays a central role in regulating eukaryotic cell-cycle progression. Through its association with $G_1$ and S phase CDK complexes it regulates activation of the retinoblastoma protein (pRb) and E2F transcription factors. Thus, selective blockade of the cyclin recruitment site would prevent recognition and subsequent phosphorylation of CDK substrates, and therefore offers a therapeutic approach towards restoration of $p21^{Waf1}$ like tumor suppression. Recently the octapeptide, HSKRRLIF, located C-terminal in $p21^{Waf1}$ which has been shown to display potent cyclic inhibitory activity due to its capacity to bind to the cyclic recruitment site. These proteins $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and $p16^{Ink4/MTS1}$, when bound to CDK complexes, inhibit their kinase activity, thereby inhibiting progression through the cell cycle (Chen, Y.P. et al. *Proc. Natl. Acad. Sci.* USA 1999, 96, 4325-29; Zheleva, D.I. et al. *J. Peptide Res.* 2002, 60, 257-270; Atkinson, G.E. et al. *Bioorg. Med. Chem. Lett.* 2002,12,

2501-2505; McInnes, C. et al. *Curr. Med. Chem.-Anticancer Agents* 2003, 3, 57-69.

**[0007]** There is therefore a need for compounds that can target the function of cell cycle suppressors such as $p27^{Kip1}$ and $p21^{Waf1/Cip1}$.

**[0008]** **SUMMARY OF THE INVENTION**

**[0009]** The present invention relates to new rapamycin derivatives for the inhibition of cell proliferation. The compounds advantageously combine two molecular functionalities that can target the functions of two or more proteins in dividing cells and interfere with cell cycle.

**[0010]** In one embodiment of the invention there is provided derivatives of rapamycin in which the 42 position of rapamycin is linked to an amino acid, or an amino alcohol, or a peptide through a carbamate ester linkage. These rapamycin derivatives can be synthesized by reacting 42-O-(4-Nitrophenoxycarbonyl)rapamycin and an amino acid, or amino alcohol, or an amino peptide under basic conditions.

**[0011]** In a further embodiment the rapamycin derivatives can be used to inhibit the cell cycle and are therefore useful for treating cell proliferation disorders.

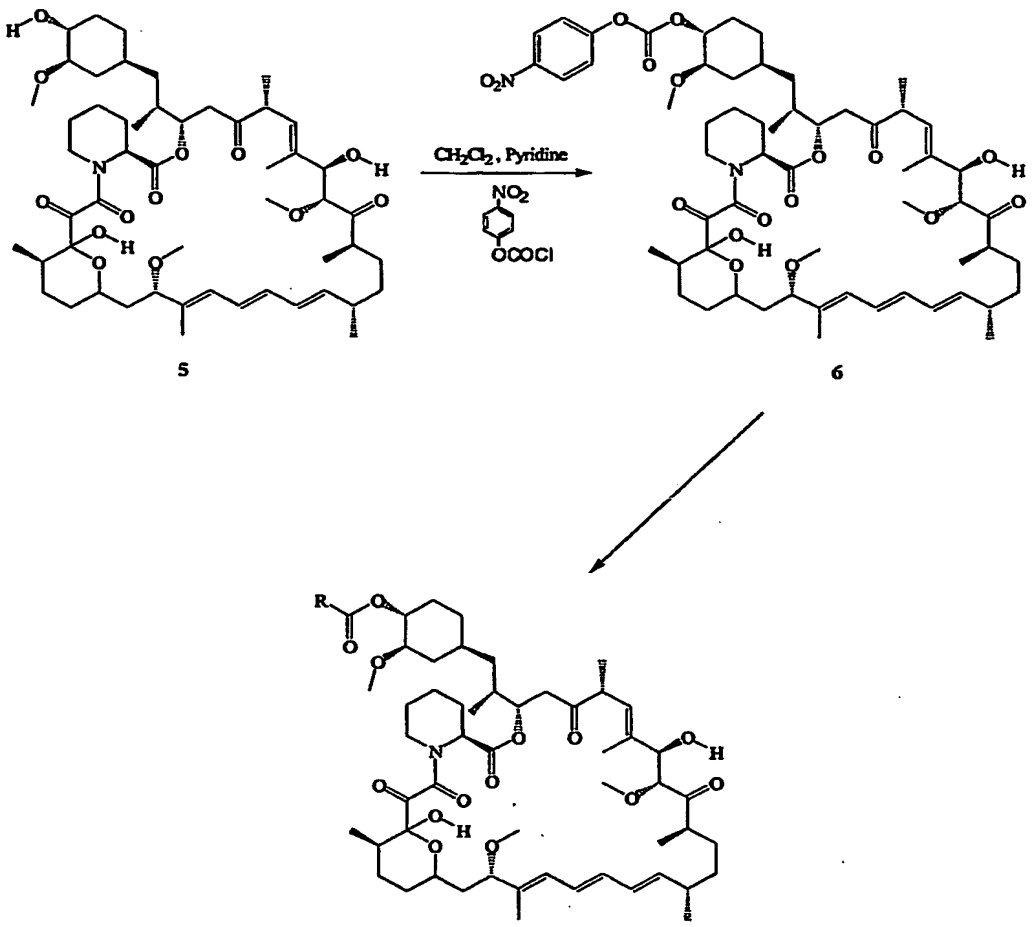
**[0012]** Further features and advantages of the present invention will become apparent from the following detailed description.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

**[0013]** This invention relates to the synthesis of novel rapamycin derivatives compounds useful for the inhibition of cell division for the treatment of diseases in which the inhibition of cell proliferation is desirable.

**[0014]** In one embodiment, amino acids and/or small peptides derivatives of the octapeptide HSKRRLIF are conjugated with rapamycin (formula **5**). The regioselective synthesis of derivatives of rapamycin **5** at the 42 position, is achieved by conjugating the amino end of the amino acids and/or active peptides with 42-O-(4-Nitrophenoxycarbonyl) rapamycin (**6**). Compounds of general formula **7** (**Scheme 1**) are thereby obtained.

**Scheme 1**

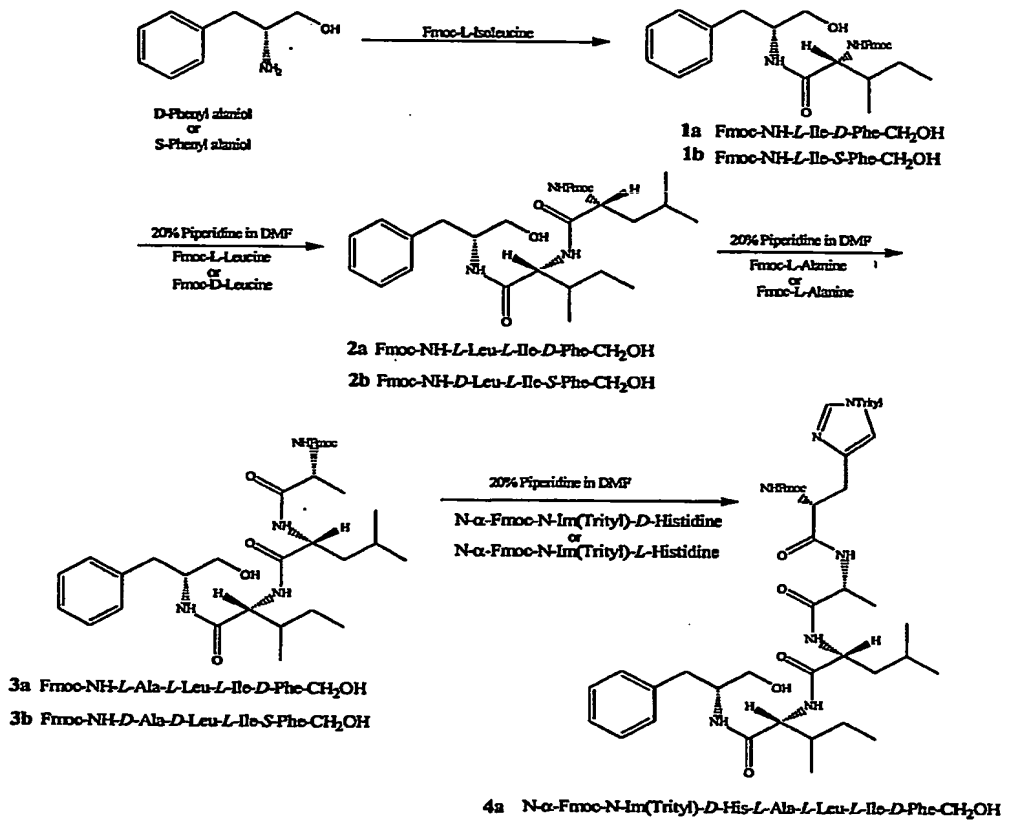



**[0015]**

**[0016]** The peptides conjugated to rapamycin preferably comprise amino acids from the C-terminal of the octapeptide HSKRRLIF. The amino acids at the N-terminal may differ from that of the octapeptide. Single amino acids may also be used. Examples of compounds obtained by the combination of 42-O-(4-Nitrophenoxycarbonyl) rapamycin and amino acids and/or peptides are given below (compounds 7a to 7u).

**[0017]** The peptides used to derive 42-O-(4-Nitrophenoxycarbonyl) rapamycin can be synthesized from amino alcohols. The first amino acid is kept as Phe-OH (or 2-amino-3-phenyl-propanol) and performing chain elongation with Fmoc chemistry in solution phase (**Scheme 2**) using DCC/HOBt as the coupling reagents.

**Scheme 2**

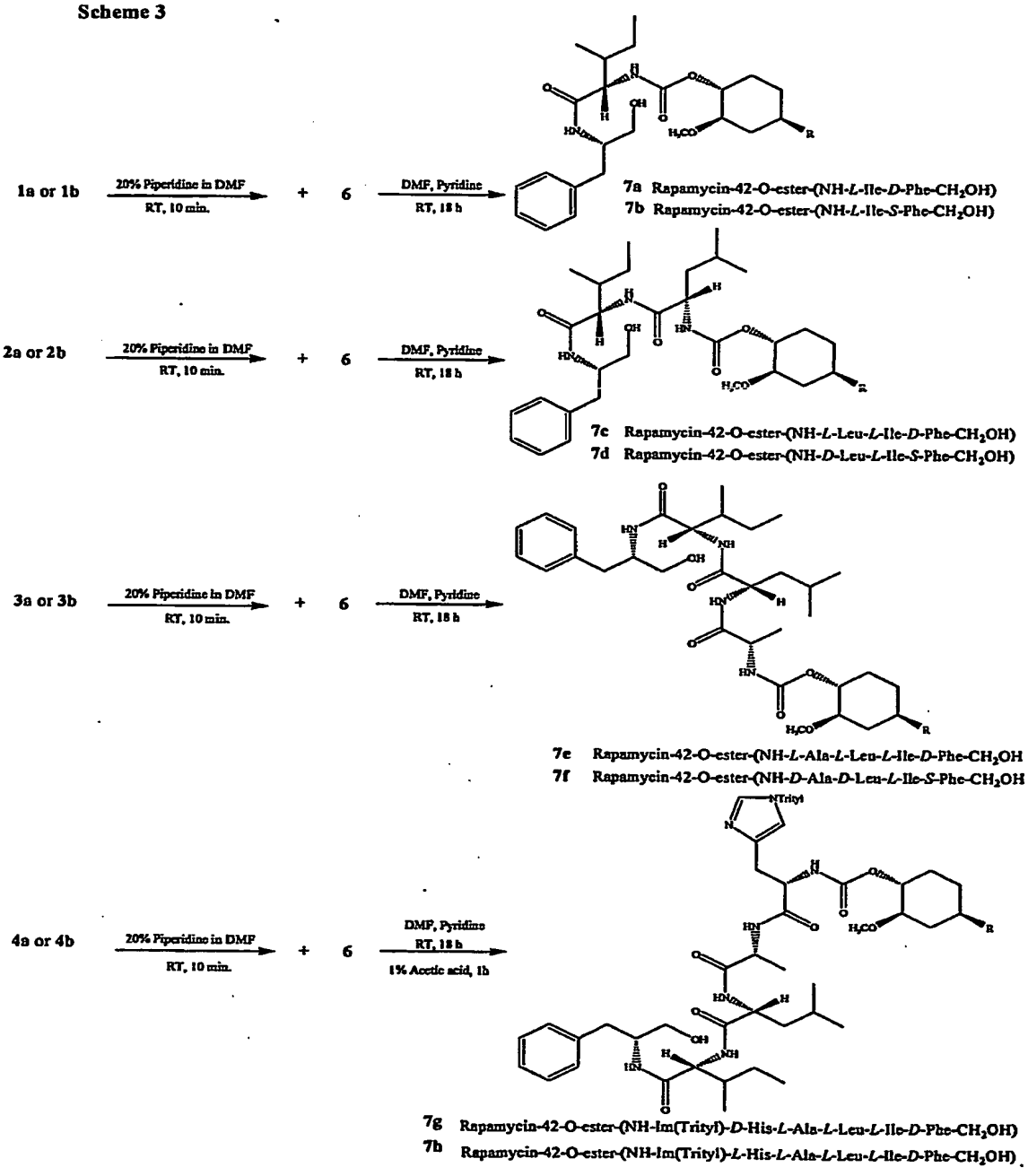



**[0018]**

**[0019]** The subsequent coupling of the peptide sequence with 42-O-(4-Nitrophenoxycarbonyl) rapamycin is done first by de-blocking the Fmoc group under basic conditions (using piperidine for example) and then by coupling the peptide with 42-O-(4-nitrophenoxy carbonyl) rapamycin (6) under

basic conditions as shown in **scheme 3** to obtain compounds of general formula **7**.

**Scheme 3**




**7a** Rapamycin-42-O-ester-(NH-*L*-Ile-*D*-Phe-$CH_2OH$)
**7b** Rapamycin-42-O-ester-(NH-*L*-Ile-*S*-Phe-$CH_2OH$)

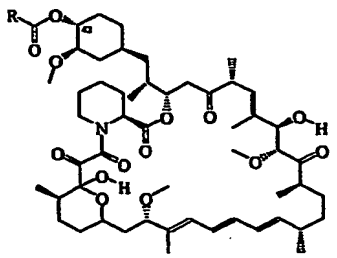
**7c** Rapamycin-42-O-ester-(NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$)
**7d** Rapamycin-42-O-ester-(NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$)

**7e** Rapamycin-42-O-ester-(NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$
**7f** Rapamycin-42-O-ester-(NH-*D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$

**7g** Rapamycin-42-O-ester-(NH-Im(Trityl)-*D*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$)
**7h** Rapamycin-42-O-ester-(NH-Im(Trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$)

**[0020]** The derivatives of rapamycin at the 42 position may also be synthesized by conjugating the amino end of amino alcohols. Compounds 7j to 7u are examples of such amino alcohols-rapamycin conjugates(**Sheet 1**).

Sheet 1



7

7a NH-*L*-Ile-*D*-Phe-$CH_2OH$

7b NH-*L*-Ile-*S*-Phe-$CH_2OH$

7c NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$

7d NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$

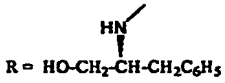
7e NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$

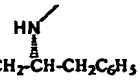
7f NH-*D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$

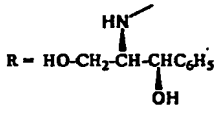
7g NH-N-Im(Trityl)*D*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$

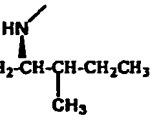
7h NH-N-Im(Trityl)*D*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$

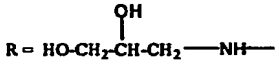
7i NH-*L*-His-*L*-Ala-*L*-Lys-*L*-Arg-*L*-Arg-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$

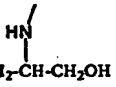
7j 

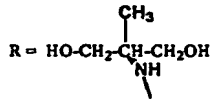
7k 

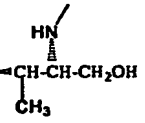
7l 

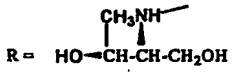
7m 

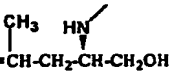
7n 

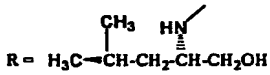
7o 

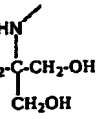
7p 

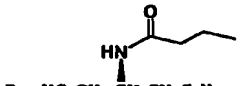
7q 

7r 

7s 

7t 

7u 

7v 

**[0021]**

**[0022]** The conjugations of rapamycin with amino alcohols or peptides comprising an amino alcohol at the "C" terminal of the peptide provides increased hydrophilic character to the compound by virtue of the presence of the free hydroxyl group.

**[0023]** The resulting compounds were screened on a panel of nine human tumor cell lines as listed in **Table 1**. Quantification of cell proliferation and cell viability was determined by measuring the amount of radioactive [$^3$H-methyl]-thymidine incorporated into DNA. The detailed experimental procedure is further described below.

[0024]

Table 1 Inhibition of $H^3$ Thymidine uptake ($IC_{50}$ in nM)

| Cell Lines | A431 | Lncap | LS174T | MCF-7 | OVCAR-3 | SKMEL-2 | SK-N-SH | SKOV-3 | D341 |
|---|---|---|---|---|---|---|---|---|---|
| Compound Adriamycin | 50.42 | 2.544 | 4.036 | 3.11 | 4.773 | 1.93 | 0.8959 | 129.7 | 7.016 |
| 5 | 341.2 | 30.33 | 979.6 | 0.4077 | 44.55 | 1.122 | 1.329 | 0.3916 | 1.561 |
| 7a | 607.9 | 153.6 | 625.6 | 29.61 | 415.9 | 78.06 | 86.93 | 80.02 | 138.2 |
| 7b | 1838 | 308.6 | 1485 | 53.32 | 423.6 | 325.5 | 143.6 | 135.5 | 182.5 |
| 7c | 286.4 | 211.8 | 1698 | 32.3 | 260.1 | 87.37 | 110.8 | 72.12 | 113.6 |
| 7d | 12748 | 197.9 | NE | 52.18 | 343.1 | 358.1 | 137.2 | 94.35 | 139.6 |
| 7e | 489.8 | 190.6 | 145.6 | 66.89 | 503.1 | 155.3 | 90.01 | 76.58 | 82.75 |
| 7f | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 7g | NE | NE | 3684 | 2437 | NE | 945.9 | NE | 595.1 | 936.7 |
| 7h | 2372 | 374.1 | 777 | 85.45 | 374.9 | 415.6 | 222.6 | 154.7 | 405.3 |
| 7i | 28361 | NE | NE | 1726 | 1667 | NE | NE | 13150 | 7717 |
| 7j | 505.7 | 166.3 | NE | 15.31 | 263.9 | 156.6 | 80.04 | 55.86 | 106.4 |
| 7k | 550.4 | 145.8 | 1380 | 15.94 | 295.9 | 109.2 | 71.32 | 66.03 | 259.4 |
| 7l | 216.8 | 64.62 | 23500 | 6.353 | 112.9 | 29.07 | 15.63 | 17.15 | 27.6 |
| 7m | 625.8 | 135.9 | 664 | 31.31 | 488.3 | 147.4 | 56.69 | 69.12 | 86.75 |
| 7n | 313.5 | 42.74 | 55081 | 2.5333 | 71.86 | 11.72 | 7.271 | 7.96 | 14.71 |
| 7o | 149.5 | 39.64 | 1027 | 2.579 | 80.73 | 48.34 | 5.837 | 27.44 | 36.16 |
| 7p | 254.3 | 7.119 | 2308 | 1.173 | 79.33 | 17.66 | 7.187 | 8.797 | 10.16 |
| 7q | 312.8 | 17.37 | 1631 | 1.291 | 56.47 | 9.154 | 5.253 | 6.346 | 25.21 |
| 7r | 190.8 | 21.76 | 1406 | 1.86 | 47.23 | 8.318 | 4.839 | 6.443 | 5.503 |
| 7s | 625 | 189 | NE | 15.59 | 355.4 | 153.9 | 97.29 | 93.64 | 103 |
| 7t | 277.9 | 202.4 | 23978 | 53.26 | 366.7 | 73.15 | 99.33 | 64.12 | 61.17 |
| 7u | 282.3 | 13.98 | 1594 | 1.539 | 56.62 | 6.152 | 6.402 | 8.078 | 20.83 |
| 7v | 1032 | 48.34 | NE | 5.522 | 95.46 | 81.01 | 18.78 | 10.36 | 15 |

**[0025]** As can be seen the compounds can be at least as efficient as rapamycin or in some instances the compounds are more efficient than rapamycin. Without wishing to be bound by theory, the conjugation of peptides and/or amino acids or amino alcohols to rapamycin may provide a "bullet" capable of inactivating the functions of two or more proteins, such as $p27^{Kip1}$ and $p21^{Waf1/Cip1}$. This capacity to dual functional inactivation can be advantageous in cases where one of the target becomes resistant such as by mutation for example.

**[0026]** Thus the peptide and amino acid derivatives of rapamycin of the present invention are useful for the treatment of conditions in which the control or inhibition of the cell cycle is desirable. Such conditions may comprise but are not limited to: cancer (including solid tumors and leukemia/lymphoma), hyperplasia, psoriasis, fungal infections and the like. It will be appreciated that administration of the compounds of the present invention may be prophylactic to patients susceptible to the above mentioned conditions. It will also be appreciated that the compounds of the present invention may also be used to treat or prevent hyperproliferative vascular disorders such as restenosis. In particular, the compounds may be applied to, or associated with, surgical stents to prevent restenosis at the site of the stent application in blood vessels. The compounds may for example be incorporated in drug-eluting stents. It will be appreciated that the compounds may also be administered to patients already having stents or about to receive such stents.

[0027] Preferred routes for the administration of the compounds of the present invention are intravenous, intramuscular, subcutaneous, intraperitoneous, intraarterial, and oral. It will be appreciated that other methods of administration, as would be known to one skilled in the art, may be used such as, for example, local administration at the site of a tumor using a catheter.

[0028] The compounds are preferably administered as part of a pharmaceutical composition which may also comprise a pharmaceutically acceptable carrier as would be obvious to one skilled in the art.

[0029] In another embodiment the compounds may be useful as immunosuppressants and can therefore be useful in treating diseases related to undesired immune responses. Non-limiting example includes preventing graft rejections (host vs graft disease, graft vs host disease), diseases of inflammation and autoimmune diseases such as arthritis.

[0030] **Screening procedure**

[0031] 1. Cell culture: For each cell line, culture according to the ATCC Product Information Sheet provided. Cell lines are always freshly thawed prior to each experiment. For all experiments exponentially growing cells are harvested and centrifuged at 1100 rpm, the spent medium is aspirated and cell pellets are resuspended in fresh complete medium. Viable cells are enumerated by trypan blue exclusion using a hemacytometer.

[0032] 2. Cells are then seeded in 96 well tissue culture plates in a total volume of 100 µL/well. A preliminary experiment should be performed to determine the most

appropriate cell density for each individual cell line. Cells are allowed to attach and acclimate overnight.

**[0033]** 3. Addition of test compounds: Each test compound is dissolved in DMSO at a final concentration of 2 mM.Each stock compound is then diluted in complete medium (1:100) to obtain a 20 μM working solution. The working solution is used for further serial dilutions to obtain concentrations of 200 nM, 20 nM, 2 nM, .2 nM and .02 nM. 100 μL of each dilution is added to the 100 μL cell cultures (3 replicates), to give final test concentrations of 100 nM, 10 nM, 1 nM, .1 nM and .01 nM. Using this system of dilutions, the maximum concentration of DMSO to which the cells are exposed will be 0.01% v/v. Therefore, 0.01% DMSO will be added to control cells to which no test compounds have been added. On each plate we will also include two positive controls: Adriamycin Hydrochloride and Rapamycin. For each positive control choose 3 concentrations in a range that will achieve an $LC_{50}$, this range will be cell line specific and must be predetermined in a pilot experiment. Plates are incubated for 96 hours prior to harvesting. [$^3$H-methyl]-thymidine incorporation: After 80 hours incubation, add 10 μL (0.5 μCi) of [$^3$H-methyl]-thymidine diluted in 1X HBSS. Incubate plates overnight.

**[0034]** Remove growth medium from each well and add 100 uL of Trypsin-EDTA. Incubate plate at 37°C until cells have been trypsinized (check under microscope). Harvest the detached cells using a semiautomatic cell harvester. Dry filters prior to addition to scintillation vials. Automatically dispense 2 mL of scintillation fluid into each vial. Cap vials and count on program 1 ($^3$H, 1 min, DPM).

**[0035]** The average and standard error from the DPM counts of replicate samples are calculated. The $IC_{50}$ values of these screening results are listed in **Tabl 1.**

**[0036]** EXAMPLE 1

**[0037]** **Synthesis of Fmoc-NH-*L*-Ile-*D*-Phe-$CH_2OH$ (1a)**

**[0038]** (R)(+)-2-amino-3-phenyl-1-propanol (427 mg, 2.83 mmol) dissolved in dry DMF (20 mL) , stirred under nitrogen, to this stirred mixture at 25°C DCC (699 mg, 3.39 mmol), HOBt (457 mg, 3.38 mmol) was added with constant stirring. After 10 minutes of stirring N-(9-fluorenylmethoxycarbonyl)-*L*-isoleucine (1.0g, 2.83 mmol) was added to the above mixture and then stirred at 25°C for 14 h. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (2% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.6), LC/MS also showed molecular ion peak corresponding to the dipeptide **1a** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 2% MeOH: $CH_2Cl_2$ to give 650 mg of the dipeptide Fmoc-NH-*L*-Ile-*D*-Phe-$CH_2OH$ (**1a**) as a white solid. Checked on LC/MS which showed $M^+$+1 (487.4) and $M^+$+ Na (509.2).

**[0039]** EXAMPLE 2

**[0040]** **Synthesis of Fmoc-NH-*L*-Ile-*S*-Phe-$CH_2OH$ (1b)**

**[0041]** (S)(+)-2-amino-3-phenyl-1-propanol (1.3g mg, 8.59 mmol) dissolved in dry DMF (100 mL) , stirred under nitrogen, to this stirred mixture at 25°C DCC (1.94g, 10.9 mmol), HOBt (1.27g, 10.9 mmol) was added with constant stirring. After 10 minutes of stirring N-(9-fluorenylmethoxycarbonyl)-*L*-isoleucine (3.0g, 2.83 mmol)

was added to the above mixture and then stirred at 25°C for 14 h. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (2% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.6), LC/MS also showed molecular ion peak corresponding to the dipeptide **1b** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 2% MeOH: $CH_2Cl_2$ to give 2.8g of the dipeptide Fmoc-NH-*L*-Ile-*S*-Phe-$CH_2OH$ (**1b**) as a white solid. Checked on LC/MS which showed $M^+$+1 (487.2) and $M^+$+ Na (509.2).

**[0042]** EXAMPLE 3

**[0043]** **Synthesis of Fmoc-NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (2a)**

**[0044]** Dipeptide Fmoc-NH-*L*-Ile-*D*-Phe-$CH_2OH$ (1a) (520 mg, 1.06 mmol) was taken in 20% piperidine in DMF (2 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (263.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x10 mL), the free amino dipeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (2 mL) and added to the mixture of N-(9-fluorenylmethoxycarbonyl)-*L*-leucine (38.1 mg, 1.06 mmol), DCC (262.0 mg, 1.27 mmol) and HOBt (171.4 mg, 1.27 mmol) in dry DMF (20 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (3% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.5), LC/MS also showed molecular ion peak

corresponding to the tripeptide **2a** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 3% MeOH: $CH_2Cl_2$ to give 310 mg of the tripeptide Fmoc-NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**2a**) as a white solid. Checked on LC/MS which showed $M^+$+1 (600.3) and $M^+$+ Na (622.3).

**[0045]** EXAMPLE 4

**[0046]** **Synthesis of Fmoc-NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$ (2b)**

**[0047]** Dipeptide Fmoc-NH-*L*-Ile-*S*-Phe-$CH_2OH$ (**1b**) (2.0g, 4.11 mmol) was taken in 20% piperidine in DMF (20 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ile-*S*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (263.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x20 mL), the free amino dipeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (5 mL) and added to the mixture of N-(9-fluorenylmethoxycarbonyl)-*D*-leucine (1.59g, 4.52 mmol), DCC (931 mg, 4.52 mmol) and HOBt (610.0 mg, 4.52 mmol) in dry DMF (150 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (5% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.5), LC/MS also showed molecular ion peak corresponding to the tripeptide **2b** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 4% MeOH: $CH_2Cl_2$ to give 450 mg of the tripeptide Fmoc-NH-*D*-Leu-*L*-

Ile-*S*-Phe-$CH_2OH$ (**2b**) as a white solid. Checked on LC/MS which showed $M^+$+1 (600.3) and $M^+$+ Na (622.3).

**[0048]** EXAMPLE 5

**[0049]** **Synthesis of Fmoc-NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (3a)**

**[0050]** Tripeptide Fmoc-NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**2a**) (75 mg, 0.125 mmol) was taken in 20% piperidine in DMF (0.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (376.1) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2 mL), the free amino tripeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (0.5 mL) and added to the mixture of N-(9-fluorenylmethoxycarbonyl)-*L*-alanine (42.0 mg, 0.137 mmol), DCC (28.0 mg, 0.137 mmol) and HOBt (18.4 mg, 0.137 mmol) in dry DMF (2.5 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (10% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.45), LC/MS also showed molecular ion peak corresponding to the tetrapeptide **3a** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 8% MeOH: $CH_2Cl_2$ to give 70 mg of the tetrapeptide Fmoc-NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**3a**) as a white solid. Checked on LC/MS which showed $M^+$+1 (671.3) and $M^+$+ Na (693.3).

**[0051]** EXAMPLE 6

**[0052]** **Synthesis of Fmoc-NH-*D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$ (3b)**

**[0053]** Tripeptide Fmoc-NH-*D*-Leu-*D*-Ile-*S*-Phe-$CH_2OH$ (2b) (330 mg, 0.550 mmol) was taken in 20% piperidine in DMF (1.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*D*-Leu-*D*-Ile-*S*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed M-1 (376.1) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5 mL), the free amino tripeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.0 mL) and added to the mixture of N-(9-fluorenylmethoxycarbonyl)-*D*-alanine (188.4 mg, 0.606 mmol), DCC (124.0 mg, 0.606 mmol) and HOBt (81 mg, 0.606 mmol) in dry DMF (2.5 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (10% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.40), LC/MS also showed molecular ion peak corresponding to the tetrapeptide **3b** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 7% MeOH: $CH_2Cl_2$ to give 40 mg of the tetrapeptide Fmoc-NH-*D*-Ala-*D*-Leu-*D*-Ile-*S*-Phe-$CH_2OH$ (**3b**) as a white solid. Checked on LC/MS which showed $M^+$+1 (671.4) and $M^+$+ Na (693.4).

**[0054]** EXAMPLE 7

**[0055]** **Synthesis of N-α-Fmoc-N-Im(trityl)-D-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (4a)**

**[0056]** Tetrapeptide Fmoc-NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (3a) (300 mg, 0.447 mmol) was taken in 20% piperidine in DMF (2.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$+1 (450.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5 mL), the free amino tetrapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of N- α -(9-fluorenylmethoxycarbonyl)-N-Im(trityl)-*D*-histidine (304.8 mg, 0.492 mmol), DCC (101.3 mg, 0.492 mmol) and HOBt (66.4 mg, 0.492 mmol) in dry DMF (2.5 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (15% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.55), LC/MS also showed molecular ion peak corresponding to the pentapeptide **4a** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 10% MeOH: $CH_2Cl_2$ to give 376 mg of the pentapeptide N-α-Fmoc-N-Im(trityl)-*D*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**4a**) as white solid. Checked on LC/MS which showed $M^+$+1 (1050.6).

**[0057]** EXAMPLE 8

**[0058]** **Synthesis of N-α-Fmoc-N-Im(trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (4b)**

**[0059]** Tetrapeptide Fmoc-NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (3a) (300 mg, 0.447 mmol) was taken in 20% piperidine in DMF (2.5 mL) and stirred for 15 minutes at 25°C, TLC

examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$+1 (450.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5 mL), the free amino tetrapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of N- α -(9-fluorenylmethoxycarbonyl)-N-Im(trityl)-*L*-histidine (304.8 mg, 0.492 mmol), DCC (101.3 mg, 0.492 mmol) and HOBt (66.4 mg, 0.492 mmol) in dry DMF (2.5 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h white colored crystals precipitated out (DCU), which was filtered. The filterate was checked on TLC (15% MeOH: $CH_2Cl_2$) which showed formation of a new compound at higher $R_f$ (0.55), LC/MS also showed molecular ion peak corresponding to the pentapeptide **4b** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with 10% MeOH: $CH_2Cl_2$ to give 376 mg of the pentapeptide N-α-Fmoc-N-Im(trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**4b**) as white solid. Checked on LC/MS which showed $M^+$+1 (1050.6).

**[0060]** EXAMPLE 9

**[0061]** **42-O-(4-Nitrophenoxycarbonyl) rapamycin (6)**

**[0062]** To a solution of 5.00 g (5.47 mmol) of rapamycin (5) in 40 ml of dichloromethane cooled at -78°C with dry ice and acetone bath was added 650 μl of dry pyridine and 1.65 g of p-nitrophenyl chloroformate dissolved in 10 ml of dichloromethane. The reaction mixture was allowed to warm to ambient temperature and stirred for two hours under nitrogen. After two hours 325 μl of dry pyridine and 555 mg

of the p-nitrophenyl chloroformate was added to the above reaction mixture. The reaction mixture was stirred under nitrogen for 18h. The progress of the reaction was monitored by mass spectrum. After 18h the reaction mixture was concentrated in vacuum and partitioned between ether and water. The organic phase was washed with 0.1N HCl (3x) than with saturated brine solution (2x), dried over sodium sulphate, filtered and concentrated in vacuum to give the pale yellow solid, which was purified on silica gel (Silica gel 60, 63-200μ). Elution with 40% and then 50% ethyl acetate: Hexane gave 4.7 g of the title compound (6) as yellow solid.

**[0063]** $^1H$ NMR ($CDCl_3$): δ 8.27 and 7.39 (aromatic-H, 4H), 4.63 (42C, 1H): Mass spectra: Positive M + Na 1101.5 (100%): Negative M-1 1077.5 (100%).

**[0064]** EXAMPLE 10

**[0065]** **Synthesis of Rapamycin- 42-O-ester-(NH-*L*-Ile-*D*-Phe-$CH_2OH$) (7a)**

**[0066]** Dipeptide Fmoc-NH-*L*-Ile-*D*-Phe-$CH_2OH$ (**1a**) (67.0 mg, 0.138 mmol) was taken in 20% piperidine in DMF (0.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (263.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2.5 mL), the free amino dipeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (2 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl)rapamycin (6) (100 mg, 0.092 mmol) and pyridine (50 μL)in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at

25°C. After 14 h reaction mixture was checked on TLC (pure ethyl acetate) which showed formation of a new compound at lower $R_f$ (0.5) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7a** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with pure ethyl acetate to afford 50 mg (45% yield) of the conjugate Rapamycin-42-O-ester-(NH-*L*-Ile-*D*-Phe-$CH_2OH$) **7a** as a light yellow colored solid. Checked on LC/MS which showed M-1 1202.7 (100%).

**[0067]** EXAMPLE 11

**[0068]** **Synthesis of Rapamycin- 42-O-ester-(NH-*L*-Ile-*S*-Phe-$CH_2OH$) (7b)**

**[0069]** Dipeptide Fmoc-NH-*L*-Ile-*S*-Phe-$CH_2OH$ (**1b**) (67.0 mg, 0.138 mmol) was taken in 20% piperidine in DMF (0.2 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ile-*S*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (263.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2.5 mL), the free amino dipeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (2 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl)rapamycin (**6**) (100 mg, 0.092 mmol) and pyridine (50 μL)in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (pure ethyl acetate) which showed formation of a new compound at lower $R_f$ (0.4) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7b** with other impurities peaks. The crude product

was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with pure ethyl acetate to afford 18.8 mg (20% yield) of the conjugate Rapamycin-42-O-ester-(NH-*L*-Ile-*S*-Phe-$CH_2OH$) **7b** as a light yellow colored solid. Checked on LC/MS which showed M-1 1202.6 (100%).

**[0070]** EXAMPLE 12

**[0071]** **Synthesis of Rapamycin- 42-O-ester-(NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$) (7c)**

**[0072]** Dipeptide Fmoc-NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (2a) (60.6 mg, 0.101 mmol) was taken in 20% piperidine in DMF (0.3 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (376.1) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2.5 mL), the free amino tripeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (2 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl)rapamycin (6) (100 mg, 0.092 mmol) and pyridine (50 μL)in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (pure ethyl acetate) which showed formation of a new compound at lower $R_f$ (0.5) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product 7c with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200μ) column and eluted with pure ethyl acetate to afford 45 mg (37% yield) of the conjugate Rapamycin-42-O-ester-(NH-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$)

**7c** as a white solid. Checked on LC/MS which showed M-1 1315.6 (100%).

**[0073]** EXAMPLE 13

**[0074]** **Synthesis of Rapamycin- 42-O-ester-(NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$) (7d)**

**[0075]** Dipeptide Fmoc-NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$ **(2b)** (60.6 mg, 0.101 mmol) was taken in 20% piperidine in DMF (1.0 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$-1 (376.1) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5.0 mL), the free amino tripeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (2 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) (100 mg, 0.092 mmol) and pyridine (50 µL) in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (pure ethyl acetate) which showed formation of a new compound at lower $R_f$ (0.45) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7d** with other impurities peaks. The crude product was chromatographed on silica gel (Silica gel 60, 63-200µ) column and eluted with pure ethyl acetate to afford 44 mg (36% yield) of the conjugate Rapamycin-42-O-ester-(NH-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$) **7d** as a white solid. Checked on LC/MS which showed M-1 1315.6 (100%).

**[0076]** EXAMPLE 14

**[0077] Synthesis of Rapamycin- 42-O-ester-(NH- *L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$) (7e)**

**[0078]** Tetrapeptide Fmoc-NH-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (3a) (60 mg, 0.089 mmol) was taken in 20% piperidine in DMF (1.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$+1 (450.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2.5 mL), the free amino tetrapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) (100 mg, 0.092 mmol) and pyridine (50 µL) in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (5% MeOH:$CH_2Cl_2$) which showed formation of a new compound at lower $R_f$ (0.45) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product 7e with other impurities peaks. The crude product was purified on preparative TLC using 5% MeOH: $CH_2Cl_2$ as the developing solvent system to afford 9.8mg (10% yield) of the conjugate Rapamycin-42-O-ester-(NH- *L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$) 7e as a white solid. Checked on LC/MS which showed $M^+$+Na 1410.8 (100%).

**[0079]** EXAMPLE 15

**[0080] Synthesis of Rapamycin- 42-O-ester-(NH- *D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$) (7f)**

**[0081]** Tetrapeptide Fmoc-NH-*D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$ (3b) (60 mg, 0.089 mmol) was taken in 20% piperidine in DMF

(1.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-*D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$+1 (450.3) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x2.5 mL), the free amino tetrapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) (100 mg, 0.092 mmol) and pyridine (50 µL) in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (5% MeOH:$CH_2Cl_2$) which showed formation of a new compound at lower $R_f$ (0.45) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7f** with other impurities peaks. The crude product was purified on preparative TLC using 5% MeOH: $CH_2Cl_2$ as the developing solvent system to afford 12.3 mg (11%yield) of the conjugate Rapamycin-42-O-ester-(NH- *D*-Ala-*D*-Leu-*L*-Ile-*S*-Phe-$CH_2OH$) **7f** as a white solid. Checked on LC/MS which showed $M^+$+Na 1410.8 (100%).

**[0082]** EXAMPLE 16

**[0083] Synthesis of Rapamycin- 42-O-ester-(NH-N-(Trityl)-D-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (7g)**

**[0084]** Pentapeptide N-α-Fmoc-N-Im(trityl)*D*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (4) (150 mg, 0.142 mmol) was taken in 20% piperidine in DMF (2.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-N-Im(trityl)-D-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination,

which showed $M^+$+1 (828) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5 mL), the free amino pentapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) (158 mg, 0.147 mmol) and pyridine (50 µL) in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (5% MeOH:$CH_2Cl_2$) which showed formation of a new compound at lower $R_f$ (0.45) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7g** with other impurities peaks The crude product was chromatographed on silica gel (Silica gel 60, 63-200µ) column and eluted with pure 10% MeOH: $CH_2Cl_2$ to afford 41 mg (16% yield) of the conjugate Rapamycin-42-O-ester-(NH-N-(Trityl)-D-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**7g**) as a white solid. Checked on LC/MS which showed M-1 1765.6 (90%) and 1766.8 (100%).

**[0085]** EXAMPLE 17

**[0086] Synthesis of Rapamycin- 42-O-ester-(NH-N-(Trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (7h)**

**[0087]** Pentapeptide N-α-Fmoc-N-Im(trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**4b**) (150 mg, 0.142 mmol) was taken in 20% piperidine in DMF (2.5 mL) and stirred for 15 minutes at 25°C, TLC examination showed complete removal of the Fmoc protecting group ($NH_2$-N-Im(trityl)-D-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$), further confirmed by LC/MS examination, which showed $M^+$+1 (828) peak. The reaction mixture was concentrated in vacuo, excess of the piperidine was removed by co-evaporating with toluene (2x5 mL), the

free amino pentapeptide was further dried over high vacumn for 30 minutes, and then redissolved in dry DMF (1.5 mL) and added to the mixture of 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) (158 mg, 0.147 mmol) and pyridine (50 µL) in dry DMF (10 mL) at 25°C. The stirring was further continued for 14 h at 25°C. After 14 h reaction mixture was checked on TLC (5% MeOH:$CH_2Cl_2$) which showed formation of a new compound at lower $R_f$ (0.45) then the starting material, LC/MS also showed molecular ion peak corresponding to the conjugated product **7h** with other impurities peaks The crude product was chromatographed on silica gel (Silica gel 60, 63-200µ) column and eluted with pure 10% MeOH: $CH_2Cl_2$ to afford 41 mg (16% yield) of the conjugate Rapamycin-42-O-ester-(NH-N-(Trityl)-*L*-His-*L*-Ala-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (**7h**) as a white solid. Checked on LC/MS which showed M-1 1765.6 (90%) and 1766.8 (100%).

**[0088]** EXAMPLE 18

**[0089] Synthesis of $NH_2$-N-(Trityl)-*L*-His-*L*-Ala-*L*-Lys-*L*-Arg-*L*-Arg-*L*-Leu-*L*-Ile-*D*-Phe-$CH_2OH$ (7i)**

**[0090]** Octapeptide (**7i**) was synthesized by the reported procedure (Atkinson, G.E et al. Bioorganic Med. Chem. Lett. 2002, 12, 2501-2505) using solid phase method by solid phase method on a peptide synthesizer., LCMS 1039.5379 (100%)

**[0091]** EXAMPLE 19

**[0092] Synthesis of Rapamycin-42-O-ester-(S) (-)-2-amino-3-phenyl-1-propanol (7j)**

**[0093]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (**6**) 250 mg (0.231 mmol) was dissolved in dry N, N-dimethylformamide (10 mL) and to it 50µL dry pyridine was

added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 38.5 mg (0.225 mmol) of (S) (-)-2-amino-3-phenyl-1-propanol dissolved in 1 ml of N,N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (ethyl acetate, $R_f$ 0.5) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from 10 to 50% of ethyl acetate: hexane and then pure ethyl acetate to give Rapamycin-42-O-ester-(S) (-)-2-amino-3-phenyl-1-propanol (7j) as beige colored solid 150 mg (60% yield), LC/MS showed $M^+$+ Na 1113.8 (100%) and M-1 1069.8 (100%).

**[0094]** EXAMPLE 20

**[0095] Synthesis of Rapamycin-42-O-ester-(R) (+)-2-amino-3-phenyl-1-propanol (7k)**

**[0096]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 125 mg (0.115 mmol) was dissolved in dry N, N-dimethylformamide (10 mL) and to it 50μL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 19.2 mg (0.127 mmol) of (R) (+)-2-amino-3-phenyl-1-propanol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (ethyl acetate, $R_f$ 0.6) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ)

column chromatography, by step gradient from 10 to 50% of ethyl acetate: hexane and then pure ethyl acetate to give Rapamycin-42-O-ester-(R) (+)-2-amino-3-phenyl-1-propanol (7k) as beige colored solid 65 mg (51% yield), LC/MS showed M-1 1069.8 (100%).

**[0097]** EXAMPLE 21

**[0098] Synthesis of Rapamycin-42-O-ester-(1S,2S)-(+)-2-amino-1-phenyl-1,3-propandiol (71)**

**[0099]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 150 mg (0.139 mmol) was dissolved in dry N, N-dimethylformamide (10 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 25.5 mg (0.139 mmol) of (1S,2S)-(+)-2-amino-1-phenyl-1,3-propandiol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (ethyl acetate, $R_f$ 0.4) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200µ) column chromatography, by step gradient from 10 to 50% of ethyl acetate: hexane and then pure ethyl acetate to give Rapamycin-42-O-ester-(1S,2S)-(+)-2-amino-1-phenyl-1,3-propandiol (71) as white colored solid 62.3 mg (41% yield), LC/MS showed M-1 1105.7 (100%).

**[00100]** EXAMPLE 22

**[00101] Synthesis of Rapamycin-42-O- ster-2-amino-3-methyl-1-pentanol (7m)**

**[00102]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (6 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 11.8 mg (0.101 mmol) of 2-amino-3-methyl-1-pentanol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (ethyl acetate, $R_f$ 0.6) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200µ) column chromatography, by step gradient from 10 to 50% of ethyl acetate: hexane and then pure ethyl acetate to give Rapamycin-42-O-ester-2-amino-3-methyl-1-pentanol (7m) as white colored solid 50 mg (52% yield), LC/MS showed M-1 1055.7 (100%).

**[00103]** EXAMPLE 23

**[00104] Synthesis of Rapamycin-42-O-ester-3-amino-1, 2-propanediol (7n)**

**[00105]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (5 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 9.29 mg (0.102 mmol) of 3-amino-1, 2-propanediol dissolved in 1 ml

of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.4) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 10% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-3-amino-1,2-propanediol (**7n**) as white colored solid 30 mg (31% yield), LC/MS showed M-1 1029.6 (100%).

**[00106]** EXAMPLE 24

**[00107] Synthesis of Rapamycin-42-O-ester-2-amino-1, 3-propanediol (7o)**

**[00108]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (**6**) 100 mg (0.092 mmol) was dissolved in dry N,N-dimethylformamide (5 mL) and to it 50μL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 9.29 mg (0.102 mmol) of 3-amino-1, 3-propanediol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.4) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 7% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-3-amino-1,3-propanediol (**7o**) as white colored solid 50 mg (52% yield), LC/MS showed M-1 1029.5 (100%).

**[00109]** EXAMPLE 25

**[00110] Synthesis of Rapamycin-42-O-ester-2-amino-2-methyl-1, 3-propanediol (7p)**

**[00111]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N,N-dimethylformamide (5 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 9.7 mg (0.092 mmol) of 2-amino-2-methyl-1, 3-propanediol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.6) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200µ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 7% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-2-amino-2-methyl-1,3-propanediol (7p) as white colored solid 31 mg (33% yield), LC/MS showed $M^+$+ Na 1067.5 (100%).

**[00112]** EXAMPLE 26

**[00113] Synthesis of Rapamycin-42-O-ester-(2S, 3S)-2-amino-1, 3-butanediol (7q)**

**[00114]** The active ester 42-O-(4-Nitrophenoxycarbonyl)rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N,N-dimethylformamide (20 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 9.7 mg (0.092 mmol) of (2S, 3S)-2-amino-1, 3-butanediol dissolved in 1 ml of N, N-dimethylformamide

was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.6) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 7% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-(2S, 3S)-2-amino-1, 3-butanediol (**7q**) as beige colored solid 60 mg (62% yield), LC/MS showed $M^+$+ Na 1067.7 (100%).

**[00115]** EXAMPLE 27

**[00116] Synthesis of Rapamycin-42-O-ester-(2R, 3R)-2-amino-1, 3-butanediol (7r)**

**[00117]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (**6**) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (15 mL) and to it 50μL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 9.7 mg (0.092 mmol) of (2R, 3R)-2-amino-1, 3-butanediol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.5) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 10% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-(2R, 3R)-2-amino-1, 3-butanediol (**7r**) as white colored solid 65 mg (67% yield), LC/MS showed M- 1 1043.6 (100%).

[00118] EXAMPLE 28

**[00119] Synthesis of Rapamycin-42-O-ester-(R)-(-)-2-amino-4-methyl pentanol (7s)**

[00120] The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (5 mL) and to it 50 µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 10.8 mg (0.092 mmol) of (R)-(-)-2-amino-4-methyl pentanol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.5) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200µ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 5% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-(R)-(-)-2-amino-4-methyl pentanol (7s) as white colored solid 34 mg (35% yield), LC/MS showed $M^+$ + Na 1079.7 (100%).

[00121] EXAMPLE 29

**[00122] Synthesis of Rapamycin-42-O-ester-(S)-(+)-2-amino-4-methyl pentanol (7t)**

[00123] The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (6) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (5 mL) and to it 50µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 10.8 mg (0.092 mmol) of (S)-(+)-2-amino-4-methyl pentanol dissolved in 1 ml of N, N-dimethylformamide was added over a period

of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.5) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 5% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-(S)-(+)-2-amino-4-methyl pentanol (**7t**) as white colored solid 43 mg (40% yield), LC/MS showed $M^+$ + Na 1079.7 (100%).

**[00124]** EXAMPLE 30

**[00125] Synthesis of Rapamycin-42-O-ester-Tris(hydroxymethyl)amino methane (7u)**

**[00126]** The active ester 42-O-(4-Nitrophenoxycarbonyl) rapamycin (**6**) 100 mg (0.092 mmol) was dissolved in dry N, N-dimethylformamide (5 mL) and to it 50μL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 12.35 mg (0.102 mmol) of tris(hydroxymethyl)amino methane dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (10% MeOH: $CH_2Cl_2$, $R_f$ 0.5) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200μ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 5% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-ester-tris(hydroxymethyl)amino methane (**7u**) as white colored solid 25 mg (30% yield), LC/MS showed $M^+$ + Na 1083.6 (100%).

**[00127]** EXAMPLE 31

**[00128] Synthesis of Rapamycin-42-O-(3-carboxy propanoyl) ester-(S) (-)-2-amino-3-phenyl-1-propanol (7v)**

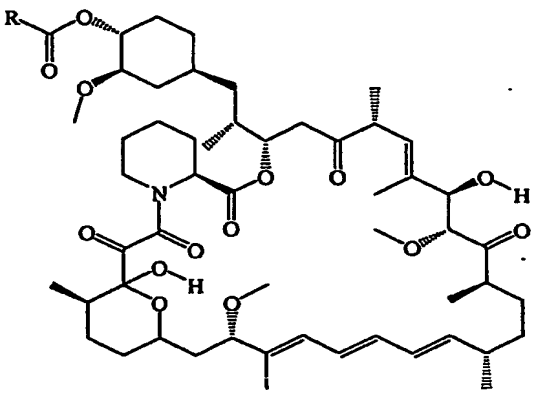
**[00129]** The active ester 42-O-(3-carboxy propanoyl) rapamycin (WO 94/24304) 75 mg (0.067 mmol) was dissolved in dry N, N-dimethylformamide (5 mL) and to it 40µL dry pyridine was added, the reaction mixture was stirred under nitrogen for five minutes at 25°C. To this stirred solution 11.1 mg (0.074 mmol) of (S) (-)-2-amino-3-phenyl-1-propanol dissolved in 1 ml of N, N-dimethylformamide was added over a period of 10 minutes with constant stirring. Stirring under nitrogen was continued for 18 h at 25°C. The progress of the reaction was monitored by TLC (5% MeOH: $CH_2Cl_2$, $R_f$ 0.6) and mass spectrum. After 18 h of the stirring the reaction mixture was evaporated to dryness. The crude mixture was purified on silica gel (Silica gel 60, 63-200µ) column chromatography, by step gradient from pure $CH_2Cl_2$ to 4% MeOH : $CH_2Cl_2$ to give Rapamycin-42-O-(3-carboxy propanoyl) ester-(S) (-)-2-amino-3-phenyl-1-propanol (7v)

**[00130]** as white colored solid 50 mg (50% yield), LC/MS showed $M^+$ + Na 1145.4 (100%).

**[00131]** The embodiment(s) of the invention described above is(are) intended to be exemplary only. The scope of the invention is therefore intended to be limited solely by the scope of the appended claims.

**I/WE CLAIM:**

1. A compound of the formula



I

Wherein

R is selected from the group comprising L-His, L-Ala, L-Lys, ,L-Arg, L-leu, L-Ile, D-Phe, D-Phe-$CH_2OH$ , D-His, D-Ala, D-Lys, D-Arg, D-Leu, D-Ile,S-Phe$Ch_2OH$, and any one of compounds 7a - 7u and wherein R and said compound of formula I are linked through a carbamate ester linkage.

2. A pharmaceutical composition comprising the compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier for use in treating cell proliferation disorders.

3. A method for treating a cell proliferation disorder comprising administering the pharmaceutical composition as claimed in claim 2 to a patient in

need thereof in an amount sufficient to reduce cell proliferation.

4. The method as claimed in claim 3 wherein said cell proliferation disorder is selected from cancer, hyperplasia, psoriasis and hyperproliferative vascular disease.

5. The method as claimed in claim 4 wherein said hyperproliferative vascular disease is restenosis.

6. A pharmaceutical composition comprising the compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier for use as an immunosuppressant.

7. A method for treating an immunological condition comprising administering the pharmaceutical composition as claimed in claim 6 to a patient in need thereof in an amount sufficient to suppress the immune system.

8. The method as claimed in claim 7 wherein said immunological disorder is selected from autoimmune disease and host-graft disease.

9. A process for the preparation of the compound of claim 1 comprising reacting 42-O-(4-Nitrophenoxycarbonyl)rapamycin and an amino acid or a peptide or an amino alcohol under basic conditions.

10. The process as claimed in claim 9 wherein said base is pyridine.

## ABSTRACT OF THE DISCLOSURE

**[00132]** The present invention relates to new rapamycin derivatives for the inhibition of cell proliferation. The compounds can advantageously target two proteins in dividing cells and interfere with cell cycle. There is thus provided derivatives of rapamycin in which the 42 position of rapamycin is linked to an amino acid or a peptide through a carbamate ester linkage. These rapamycin derivatives can be synthesized by reacting 42-O-(4-Nitrophenoxycarbonyl)rapamycin and an amino acid or a free amino peptide under basic conditions. These rapamycin derivatives can be used to inhibit the cell cycle and are therefore useful for treating cell proliferation disorders.

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